AMPH has higher potency than dopamine. These data support the hypothesis that monoamine transporter substrate-induced current couples electrically to L-type Ca^{2+} channels but not to high-voltage-activated CaV2.2 channels. Support: NIH R01 DA033930

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A Kinetic Assessment of Ligand Binding to Monoamine-Transporters

Peter S. Hasenhuetl, Michael Freissmuth, Harald H. Sitte, **Walter Sandtner**.
Dep.for Pharmacology, Medical University of Vienna, Vienna, Austria.

In this study we present a novel electrophysiological approach to measure the binding kinetics of ligands that bind to the Serotonin Transporter (SERT) and the Dopamine Transporter (DAT). The methods explored allow for the measurement of on and off-rates of drugs within a wide affinity range. Here we determined the respective rates for cocaine binding to SERT and DAT and we show that the derived kinetic parameters can very well predict cocaine affinity in equilibrium. We also explored Methyphenidat binding (licensed as Ritalin) to SERT and DAT. This drug is known to bind potently to DAT and only weakly to SERT. Our kinetic assessment revealed that the difference in the observed affinity can be solely attributed to differences in the respective on-rates of Methyphenidat, whereas the respective off-rates were similiar. Our finding therefore challenges the prevalent view that differences in potency originate from differing dissociation rates. Additionally our approach may provide guidance in the rational design of new drugs that selectively target SERT or DAT.

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Proton Transport Mechanism of the E. coli Copper Transport Efflux Pump

Arman Fathizadeh, Fatemeh Khalili-Araghi.
University of Illinois at Chicago, Chicago, IL, USA.

Gram Negative Bacteria such as E. Coli use a tripartite complex system to expel toxic chemicals, such as antibiotics, and ions out of the cell. In E.Coli, CusCBA, which is a complex of an inner membrane transporter (CusA), a connecting fusion protein (CusB), and an outer membrane channel (CusC), utilizes the proton motive force to transport copper and silver ions out of the cell. Three charged residues in the transmembrane domain have been suggested to play a crucial role in the proton transport across the membrane. The crystal structures of the complex of CusA and CusB have recently been resolved in the presence and absence of the copper ions, providing the first atomic resolution images of the assembly of the transporter and the fusion proteins in the tripartite family. Using the crystal structure of the Apo-CusBA, we have studied the proton transport mechanism in a series of unbiased molecular dynamics simulations corresponding to different protonation schemes of these three residues. The simulations have revealed two separate water permeation pathways in the transmembrane domain that coincide with the three key residues involved in the proton transport process. The presence, stability, and the number of water molecules in the two canals show a strong correlation with protonation state of the three key residues. For instance, protonation of Asp405 leads to entrance of significantly higher number of water molecules into the protein, and deprotonation of Lys984 leads to a reduction in the number of water molecules. Moreover, protonation of Asp405 in the apo state resulted in conformational changes in the transmembrane region (TM8) and the peripelasmic cleft of the pump, initiating a transition toward the Cu-bound conformation of the protein.

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The Role of Histidine Residues in the Specificity of the Human Zinc Transporter hZIP4

Robert Dempski, Sagar Antala, Elizabeth Bafaro.
Worcester Polytechnic Institute, Worcester, MA, USA.

ZIP transporters, named after the zinc regulated (Zrt) and iron regulated (Irt) transport proteins, are essential for zinc and iron translocation across cellular membranes. These proteins function to increase the cytosolic concentration of transition metals. While both zinc and iron are essential micronutrients which are required for the structure and/or function of hundreds of cellular proteins, the molecular mechanism of ZIP transporters is not well understood. Complicating mechanistic studies is the observation that the concentration of free zinc and iron is nano to picomolar. Our interest has been to elucidate the mechanism of zinc transport mediated by the human (h) ZIP4. hZIP4 is located at the primary location of zinc uptake in humans and has been directly implicated in multiple disease states including Acrodermatitis enteropathica and pancreatic cancer. However the mechanism of transport is not known. We have previously shown that zinc, nickel and copper can be transported by hZIP4, following heterologous expression in X. laevis oocytes, where there are two binding affinities (in the nM and μM range of biometal). Currently, our research interests have been to target residues of functional importance. Here, we will describe some of our recent efforts.

Genetic and Epigenetic Regulatory Systems

2331-Pos Board B468

Hybrid Microrna Control of Colon Cancer Stem Cell Asymmetric Division

Xiling Shen.
Electrical Engineering, Biomedical Engineering, Cornell University, Ithaca, NY, USA.

Colon cancer stem cells (CCSC) undergo both symmetric and asymmetric division, which balance differentiation versus self-renewal in the tumor cell population. This decision is determined by the microRNA miR-34a, whose spatial segregation generates a bimodal Notch response that determines cell fate outcomes. This bimodal response is caused by kinetic mutual sequestration between miR-34a and Notch mRNA.

However, three questions have remained since we reported the above findings (Bu et al, Cell Stem Cell, 2013). First, does miR-34a generate bimodal responses from all target genes? Second, what is the relationship between miR-34a and the canonical cell fate determinant protein Numb, which also targets Notch to regulate cell fate symmetry? And third, do these mechanisms exist in normal stem cells?

(1) A systematic study demonstrates that the kinetics of microRNA regulation is target-specific. Quantitative single-cell analysis revealed that miR-34a generates bimodal responses from a small subset of genes that are involved in cell fate determination, but regulating the majority of genes (e.g., metabolic and growth genes) in a non-bimodal manner.

(2) We report that miR-34a and Numb directly interact to form an incoherent feedforward loop. This microRNA-protein hybrid circuitry synergistically enhances Notch and cell fate asymmetry by orders of magnitude and exhibits adaptive behavior to offset interference from other miR-34a target genes, hence buffering asymmetric cell fate outcomes from fluctuations in miR-34a levels.

(3) We show that the hybrid control mechanism is active in the intestinal stem cell niche. Using innovative abdominal window and 3-D-printed intestinal insert, chronic in vivo multiphoton imaging further revealed the real-time spatiotemporal dynamics of the hybrid circuitry in live mice. This control is gradually subverted in late-stage cancer stem cells, contributing to their proliferation and malignancy.

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Nucleocytoplasmic Shuttling of a Gata-Family Transcription Factor Functions as a Development Timer

Huaqing Cai[1], Mariko Katoh-Kurasawa[2], Tetsuya Muramoto[3], Balaji Santhanam[2], Yu Long[1], Lei Li[4], Masahiro Ueda[3], Pablo A. Iglesias[5], Gad Shaulsky[2], Peter N. Devreotes[1].
[1]Cell Biology, Johns Hopkins University, Baltimore, MD, USA, [2]Baylor College of Medicine, Houston, TX, USA, [3]Osaka University, Osaka, Japan, [4]University of Virginia, Charlottesville, VA, USA, [5]Johns Hopkins University, Baltimore, MD, USA.

Biological oscillations are universal in nature, fascinating, and critical at many levels of cellular organization. In the social amoebae Dictyostelium discoideum, starvation-triggered multicellular development is orchestrated by periodic extracellular cAMP (3′-5′-cyclic adenosine monophosphate) waves, which provide both chemotactic cues and developmental signals. Repeated occupancy of the G-protein coupled cAMP receptors (cARs) promotes optimal development whereas continuous stimulation suppresses the program. While recognized nearly 40 years ago, the underlying mechanism for this intriguing stimulus-response pattern has not been elucidated. In this study, we report that a GATA family transcription factor, GtaC, which is essential for developmental progression, exhibits rapid nucleocytoplasmic shuttling in response to cAMP waves. This behavior requires coordinated action of an intrinsic nuclear localization signal (NLS) and reversible cAR-mediated phosphorylation. Disrupting GtaC shuttling by adding an exogenous NLS or mutating the residues involved in phospho-cycling leads to precocious development. Intriguingly, while cAMP is required to activate the expression of developmental genes, it also drives GtaC into the cytosol. As a result, each peak of the cAMP oscillation generates a transient burst of GtaC-dependent transcription, and the decline of cAMP allows GtaC to return to the nucleus and resensitizes the system. We demonstrate that this design, like an ''edge trigger'' logical circuit, filters out high frequency signals and counts those admitted, thereby enabling cells to modulate gene expression according to the dynamic pattern of the external stimuli.